

Appl. No.: 10/675,011
Filed: September 30, 2003
Amdt. Dated November 8, 2006

Amendments to the Claims:

Claims 1-43 (Canceled)

44. (Currently amended) A method of enhancing the expression of a biologically active polypeptide in duckweed, said method comprising culturing a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said biologically active polypeptide and wherein said biologically active polypeptide is expressed from ~~one or more nucleotide sequences~~sequences comprising a coding sequence for the biologically active polypeptide and an operably linked nucleotide sequence comprising ~~the leader~~a 5' leader sequence from ~~the~~a ribulose-bis-phosphate carboxylase small subunit (RbcS) gene of *Lemna gibba*.

45. (Currently amended) The method of claim 44, wherein said 5' leader sequence is from ~~the ribulose-bis-phosphate-carboxylase small subunit~~a RbcS gene of Lemna gibba ~~has the nucleotide sequence set forth in SEQ ID NO:16.~~

46. (New) The method of claim 45, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

47. (New) The method of claim 44, wherein said one or more nucleotide sequences have at least one attribute selected from the group consisting of:

- (a) duckweed-preferred codons in the coding sequence for said biologically active polypeptide;
- (b) a translation initiation codon that is flanked by a plant-preferred translation initiation context nucleotide sequence;
- (c) an operably linked nucleotide sequence comprising a plant intron that is inserted upstream of the coding sequence;

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(c) a signal peptide-encoding sequence operably linked to the coding sequence for said biologically active polypeptide, wherein the encoded signal peptide directs secretion of said biologically active polypeptide; and

(d) the signal peptide-encoding sequence of preceeding item (c), wherein said signal peptide-encoding sequence comprises duckweed-preferred codons.

48. (New) The method of claim 47, wherein said 5' leader sequence is from a RbcS gene of *Lemna gibba*.

49. (New) The method of claim 48, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

50. (New) The method of claim 47, wherein said biologically active polypeptide is selected from the group consisting of human growth hormone, human α -interferon, and an antibody.

51. (New) The method of claim 50, wherein said 5' leader sequence is from a RbcS gene of *Lemna gibba*.

52. (New) The method of claim 51, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

53. (New) The method of claim 47, wherein said duckweed-preferred codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons.

54. (New) The method of claim 53, wherein at least one coding sequence selected from the coding sequence for said polypeptide and the signal peptide-encoding sequence comprises between 70-100 % *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons.

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55. (New) The method of claim 47, wherein said plant-preferred translation initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to of the 5' end of the translation initiation codon.

56. (New) The method of claim 47, wherein said operably linked nucleotide sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

57. (New) The method of claim 47, wherein said signal peptide is selected from the group consisting of:

- (a) the human α -2b-interferon signal peptide;
- (b) the *Arabidopsis thaliana* chitinase signal peptide;
- (c) the rice α -amylase signal peptide;
- (d) the modified rice α -amylase signal peptide;
- (e) a duckweed signal peptide; and
- (f) a signal peptide native to the biologically active polypeptide.

58. (New) The method of claim 57, wherein said signal peptide has the amino acid sequence set forth in SEQ ID NO:6.

59. (New) The method of claim 58, wherein the coding sequence for said signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:3.

60. (New) The method of claim 44, wherein said polypeptide is human growth hormone, said human growth hormone having at least 90% sequence identity with SEQ ID NO:15.

61. (New) The method of claim 60, wherein said human growth hormone has the sequence set forth in SEQ ID NO:15.

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62. (New) The method of claim 61, wherein said coding sequence for the human growth hormone comprises the nucleotide sequence set forth in SEQ ID NO:14.

63. (New) The method of claim 60, wherein said coding sequence for the human growth hormone is operably linked to a coding sequence for a signal peptide that directs secretion of said human growth hormone, wherein said signal peptide has the amino acid sequence set forth in SEQ ID NO:13.

64. (New) The method of claim 63, wherein said coding sequence for the signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:12.

65. (New) The method of claim 60, wherein said 5' leader sequence is from a RbcS gene of *Lemna gibba*.

66. (New) The method of claim 65, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

67. (New) The method of claim 44, wherein said polypeptide is an antibody and said antibody is expressed from one or more nucleotide sequences comprising a coding sequence for a chain of the antibody.

68. (New) The method of claim 67, wherein said duckweed plant culture or duckweed nodule culture expresses and assembles the heavy chain and light chain of the antibody.

69. (New) The method of claim 67, wherein said antibody is a Fab' fragment.

70. (New) The method of claim 67, wherein said antibody is a monoclonal antibody.

71. (New) The method of claim 67, wherein said antibody is a human antibody.

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72. (New) The method of claim 67, wherein said coding sequence for said chain of said antibody is operably linked to a coding sequence for a signal peptide that directs secretion of said chain of said antibody, wherein said signal peptide has the sequence set forth in SEQ ID NO:6.

73. (New) The method of claim 72, wherein said coding sequence for the signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:3.

74. (New) The method of claim 67, wherein said 5' leader sequence is from a RbcS gene of *Lemna gibba*.

75. (New) The method of claim 74, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

76. (New) The method of claim 44, wherein said polypeptide is human α -2b-interferon having the sequence set forth in SEQ ID NO:4 or SEQ ID NO:5, or a biologically active variant thereof, wherein said variant has at least 90% amino acid sequence identity with the sequence set forth in SEQ ID NO:4 or SEQ ID NO:5.

77. (New) The method of claim 76, wherein said polypeptide is the sequence set forth in SEQ ID NO:5, and said coding sequence for said α -2b-interferon is the sequence set forth in SEQ ID NO:2.

78. (New) The method of claim 76, wherein said coding sequence for said α -2b-interferon is operably linked to a coding sequence for a signal peptide, wherein said signal peptide has the sequence set forth in SEQ ID NO:6.

79. (New) The method of claim 78, wherein the coding sequence for the signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:3.

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80. (New) The method of claim 76, wherein said 5' leader sequence is from a RbcS of *Lemna gibba*.

81. (New) The method of claim 80, wherein said 5' leader sequence has the nucleotide sequence set forth in SEQ ID NO:16.

82. (New) The stably transformed duckweed plant culture or duckweed nodule culture of claim 44.

83. (New) The stably transformed duckweed plant culture or duckweed nodule culture of claim 82, wherein said duckweed plant culture or duckweed nodule culture is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolffiella*, and genus *Lemna*.

84. (New) The stably transformed duckweed plant culture or duckweed nodule culture of claim 83, wherein said duckweed plant culture or duckweed nodule culture is selected from the group consisting of *Lemna minor*, *Lemna miniscula*, *Lemna aequinoctialis*, and *Lemna gibba*.